Jacob, Monod, the Lac Operon, and the PaJaMa Experiment—Gene Expression Circuitry Changing the Face of Cancer Research

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It is a virtually universal rule in science that if we step back to reflect upon a field currently viewed as extremely dynamic and novel, we find ourselves standing on the shoulders of those whose seminal observations gave birth to it far earlier. For those of us working in the fields of signal transduction and epigenetics within the cancer research arena, this is absolutely the case when we consider the brilliant realizations of Jacob and Monod that regulatory networks control gene expression in bacteria (1–5).

Their recognition that expression of a single gene can be repressed by another gene for response to regulatory cues from the environment ranks as one of the top, and now most heavily explored, areas of biology in general and cancer biology. A review in Cancer Research in 1961 by Pitot and Heidelberger not only pays tribute to this pivotal work of Jacob and Monod but with the prescient intent of predicting how the concepts might be woven into our understanding of carcinogenesis (6). To say their predictions were accurate would be an understatement, as is readily apparent from today’s marriage between the exploration of regulation of gene expression and our current efforts to dissect basic mechanisms underlying the origins, initiation, and progression of cancer. It becomes evident as well that the studies of Jacob and Monod, and their implications as visualized by Pitot and Heidelberger, helped usher in a biology that underpins our current efforts to dissect basic mechanisms for pathway feedback inhibition.

The nature of the trans-acting molecules through which the repressive process is mediated remained to be determined with many discussions of whether direct DNA–DNA interactions, RNA, proteins, etc., would play this role. These subsequent discussions, held at the headiest of meetings attended by many luminaries in the embryonic field of molecular biology, are credited with leading to the discovery of mRNA as put forth in a preliminary report in 1958 where it was dubbed the “PaJaMa” experiment (1, 3, 5). In this study, the investigators were able to show that a gene lac encoded a trans-acting repressor for the lac gene. In this concept, the activity of the regulator gene is demonstrated that lambda phage genes can be both induced and repressed in bacteria, the investigators established that changes in need for lactose utilization lead to negative regulation of β-galactosidase (2–5). The circuitry for this switch formed what is now famously known as the lac operon (1–5). The studies took advantage of the mating system employed in bacteria, in which the chromosomal material of the male is progressively injected over time into the female, thus progressively carrying genetic material with it. This allowed investigators to map male genes by chromosome position as their entry facilitated gene expression events in the female. Toning down the sexual connotations for the literature, the seminal study of Jacob and Monod, with participation of Arthur Pardee, was first published as a preliminary report in 1958 where it was dubbed the “PaJaMa” experiment (1, 3, 5). In this study, the investigators were able to show that a gene lac encoded a trans-acting repressor for the lac gene. In this concept, the activity of the regulator gene is induced when the repressor protein in the cytoplasm is induced by a small molecular weight product generated by the target enzyme. This circuitry paradigm contributes robustly to mechanisms for pathway feedback inhibition.

The revelations provided by Jacob and Monod started, as do many great stories in science, with a series of epiphanies by the younger investigator, Jacob, which he brought to conversations with the more established scientist, Monod. They followed their eventual joint excitement over the possibilities raised with a series of experiments, conducted during 1958 through 1961 at the Pasteur Institute in Paris. These resulted in their outlining a model for gene regulation, which survives as a core paradigm today. Their observations established the principle that to properly regulate response of an organism to changing environmental conditions, in specific bacteria for their experiments, a gene circuitry exists wherein one gene product regulates control of another gene. The result is a change in cellular phenotype for cellular metabolism (2–5). Building on experiments for demonstrating that lambda phage genes can be both induced and repressed in bacteria, the investigators established that changes in need for lactose utilization lead to negative regulation of β-galactosidase (2–5). The circuitry for this switch formed what is now famously known as the lac operon (1–5). The studies took advantage of the mating system employed in bacteria, in which the chromosomal material of the male is progressively injected over time into the female, thus progressively carrying genetic material with it. This allowed investigators to map male genes by chromosome position as their entry facilitated gene expression events in the female. Toning down the sexual connotations for the literature, the seminal study of Jacob and Monod, with participation of Arthur Pardee, was first published as a preliminary report in 1958 where it was dubbed the “PaJaMa” experiment (1, 3, 5). In this study, the investigators were able to show that a gene lac encoded a trans-acting repressor for the lac gene. In this concept, the activity of the regulator gene is induced when the repressor protein in the cytoplasm is induced by a small molecular weight product generated by the target enzyme. This circuitry paradigm contributes robustly to mechanisms for pathway feedback inhibition.

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In decades following the above observations, the paradigm of the lac operon and its constituent repressor binding to an operator and inducer ushered in an era, ever growing today, for our understanding of cellular control through signal
transduction circuity and the concepts embodied for heritability of resultant gene expression changes established by epigenetic mechanisms (2–4, 7). It has been justifiably stated that “few proteins have had such a strong impact on a field as the lac repressor has had in Molecular Biology” (2). It is hard to imagine, looking back, the degree to which the work of Jacob and Monod would become a knowledge base to build upon in elucidating the vast series of mechanisms used by cells to interpret environmental cues in processes ranging from development to those of adult cell renewing systems challenged by a myriad of normal and abnormal stimuli. A staggering portfolio of cellular machinery to implement these processes continues to unravel in what we now investigate every day as activation of, and heritably transmitting of, information from cell signaling pathways. These include switches in patterns of gene expression and the cell nuclear events that fix these gene events, including looping between DNA regions for control by gene enhancers of promoters, the roles of noncoding RNAs such as long-noncoding and miRNAs, and the roles of DNA methylation, chromatin, and nucleosome positioning in heritably locking in gene expression changes, which can all contribute to creating new cellular phenotypes (8). Indeed, one may view this as the expansion of, and definition of mechanisms for, the types of gene circuitry proposed and documented by Jacob and Monod.

With the above background in mind, it is remarkable how quickly, and with such prescience, Pitot and Heidelberger brought forth the concepts outlined in their 1961 Cancer Research review (6). The hypothesis postulated by Jacob and Monod could be transposed to a concept of induced phenotypes that are heritably perpetuated and maintained for cellular responses to short, transient exposure to carcinogens (6). They theorized that ongoing experiments in the carcinogenesis field suggested these above interactions might possibly allow engendering of a malignant cell without necessitating participation of genetic (DNA) changes such as gene mutations. Critical to this proposal, they envisioned a potential state of “reversion” which might allow for changing the malignant phenotype back to the nonmalignant state (6). These concepts are dear to the heart of researchers on the continuing quest to outline the precise roles for epigenetic alterations in the initiation and progression of cancer and the possibility that targeting such changes, and/or what controls them, could provide for potent cancer management strategies (9, 10). They perceptively weave the concepts of Jacob and Monod into a possible alternative to the then prevailing doctrine that “cancer may result from a direct interaction of carcinogen with genetic material”—a theory they reasoned had developed by “acceptance by many as the mechanism of carcinogenesis on the basis of theoretical simplicity rather than of scientific data.” As an alternative, Pitot and Heidelberger considered, and deeply modeled, how the findings of Jacob and Monod might lead to the possibility that “a cytoplasmic interaction of a carcinogen and a target protein could lead to a permanently altered and stable metabolic situation without the necessity of any direct interaction of the carcinogen and genetic material” (6). A critical feature of their hypothesis was that “under the proper circumstances and before chromosomal alterations occurred, the process might be reversed and lead to the production of a normal from a tumor cell.”

In their proposal, via a series of presented complex models, they proposed multiple scenarios and different variations of biochemical and genetic themes that could mediate their proposed interactions, arriving at the following bottom line prediction—that a carcinogen can bind to and interfere with the repressor of a growth process, thus effectively negating function of the repressor through a process of “cytoplasmic inheritance.” Thus, this interference is not dependent on continued presence of the carcinogen in daughter cells as they divide (6). Clearly, in modern parlance, we visualize these dynamics as proceeding through the cytoplasm to the nucleus via a series of signal transduction events that subsequently get abnormally fixed by epigenetic processes involving DNA methylation, chromatin, and changes in nucleosome position (8–10). Clearly, this suggests a profound role of epigenetic abnormalities early during cancer initiation and this possibility is the subject of many investigations today (9, 10). In this regard, Pitot and Heidelberger wisely articulate several key rules, and cautions, inherent to their proposed mechanisms and this wisdom enriches their predictions as they are playing out today. First, they stress that “it must be apparent to the reader that we are here dealing only with the earliest changes in carcinogenesis. Once the altered regulation is established (possibly within minutes or hours), other effects appear, such as aneuploidy, increased glycolysis, apparent multiple enzyme deletions, etc., which are probably secondary to the primary changes” (6). Second, “it is not our intention to rule out or deny the possibility that chemical carcinogenesis is a consequence of the direct interaction of the compound with genetic material. Rather, it is our purpose to call attention to alternative explanations, based upon current concepts of metabolic regulation and control, that permit the perpetuation of metabolic changes brought about by the temporary interaction of the carcinogen and a cytoplasmic protein” (6). Finally, they conclude that “by the application of these or similar theoretical models, it is possible to reconcile the large body of sound experimental data on chemical carcinogenesis with current concepts of metabolic regulation, and early cancer could be considered as a phenotypic rather than a genotypic disease” (6).

In reviewing the work of Jacob and Monod, John Beckwith (5) provides a wonderful sentiment that might serve also as a coda to the ingenious joining by Pitot and Heidelberger of the lac operon story with the field of human carcinogenesis—“new theories that become successful paradigms for their field, in their initial form at least, do not provide a correct explanation for all of the phenomena that are considered important to that field.” And, yet as implied here, any initial flaws in such theories do not prevent their never being separated from the body of invaluable work they help to spawn. Our understanding today of gene transcription is driving virtually every aspect of basic and translational tumor biology, again reminding us of our ride on the shoulders of those coming before. Their publication by Pitot and Heidelberger is, then, emblematic of why we are celebrating 75 years of publishing in Cancer Research.

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No potential conflicts of interest were disclosed.

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